REMARKS

In order to expedite the prosecution of the present application, Claims 1 and 4-15 have been canceled and replaced by newly presented Claims 16-23 which more particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specific support for Claim 16 can be found on specification page 5, lines 5-10. Support for newly presented Claim 20 is found in canceled Claim 8. No new matter has been added.

Claims 1 and 4-6 have been rejected under 35 USC 112, first paragraph, as not being enabled by the originally filed specification. It is respectfully submitted that this rejection has been made moot with the cancellation of Claims 1 and 4-6 and that currently presented Claims 16-23 clearly meet with the requirements of 35 USC 112.

Claims 1 and 4-15 have been rejected under 35 USC 103(a) as being unpatentable over Murcia et al in view of Postaire et al and Ginoux. Applicants respectfully submit that the currently presented claims clearly are patentably distinguishable over the prior art cited by the Examiner.

Currently presented Claim 16 is directed to a method of inhibiting the malignant progression of the tumor in a subject which comprises the step of administering to the subject in which the malignant progression is to be inhibited a pharmacologically effective amount of a superoxide dismutase combined with gliadin. Claim 20 is directed to a method of inhibiting the metastasis of a tumor in a subject which comprises the step of administering to the subject in which the metastasis is to be inhibited a pharmacologically effective amount of a superoxide dismutase combined with gliadin.

As pointed out previously, the instant invention is based on the discovery that superoxide dismutase combined with gliadin can function as an agent to induce host antioxidant enzymes including SOD, catalase and glutathione peroxidase,

throughout the body of the subject. Conventional SOD preparations do not affect SOD levels at any tissues and only acts by itself as an antioxidant at the site of the drug administration. That is, although it is known to orally administer SOD-containing compositions to treat certain cancers and illnesses of the digestive system, the SOD-containing composition is only orally administered in order for the SOD to contact directly with tumors located in the digestive track before inactivation of the SOD occurs. As such, it is respectfully submitted that the prior art cited by the Examiner does not disclose the presently claimed invention.

The Murcia et al reference discloses that several Mediterranean and tropical fruits have antioxidant activity. This reference has no disclosure with respect to SOD activity in that it evaluates the antioxidant or scavenger activity of different fruits, including melon pulp homogenate, by estimating activities against the hydroxyl radical, HOCl and hydrogen peroxide. No SOD activity was measured because SOD activity is measured only when dismutation activity of O_{2-} is measured. As stated previously, Applicants admit that SOD is present in melons because all plants and animals have SOD present as an essential body constituent. However, conventionally administered SOD only works locally and does not work in the body. As such, this reference contains no disclosure which would suggest to one of ordinary skill in the art that SOD combined with gliadin could be used to treat tumors throughout the body.

The Ginoux et al reference discloses a soluble *Cucumis melo* protein extract having a superoxide dismutase enzyme activity. This protein extract is said to be useful for cosmetic purposes, medical purposes (anti-cancer agents for the digestion system, antioxidant) and food purposes, (replacement of synthetic antioxidants). Although this reference discloses that the protein extract having an elevated superoxide dismutase activity can be used to treat

certain cancers of the digestive system, as explained previously, this is only because orally administered SOD contacts directly with tumors located in the digestive track before inactivation of the SOD occurs. Typically, proteinase in the digestive juices destroys SOD activity. As such, the anti-tumor effect is limited only to the digestive system which comes directly in contact with the SOD.

In contrast to the protein extract of Ginoux et al having SOD activity, the present invention discloses that SOD combined with gliadin is effective on a subcutaneously implanted mouse tumor, liver metastasis as well as prostate cancer when the SOD-gliadin composition is administered orally or parenterally. As such, the treated SOD of the present invention can be used as an effective anti-tumor agent against a tumor located anywhere in the subject's body and not just locally which contacts with the treated SOD.

The Postaire et al reference discloses pharmaceutical compositions that are suitable for the oral administration of superoxide dismutases. These compositions are made up of a combination of a superoxide dismutase and at least one lipid or protein. These pharmaceutical compositions are said to be useful in the treatment of inflammatory processes, such as rheumatism and fibrosis, viral processes, such as HIV infection, and toxic conditions associated with the presence of substantial amounts of oxygen, such as central nervous system disorders, ischemia, non-vascular gastrointestinal disorders, eye disorders or control of the undesirable effects of anti-cancer treatments. This reference discloses that a SOD composition is particularly useful for oral administration of SOD because it significantly increases the bioavailability of SOD in that it protects the SOD at an acid pH and constitutes a sustained release form. However, like the previously discussed references, there is no disclosure in Postaire et al which suggests that the treated SOD disclosed there could be used in the treatment of cancerous tumors. Therefore, Applicants respectfully submit that Postaire, in

combination with Ginoux et al and Murcia et al, does not disclose the presently claimed invention.

In the outstanding Office Action, the Examiner has responded to Applicants' position that the gliadin-coated superoxide dismutase functions by a different mechanism than uncoated superoxide dismutase by stating that the mechanism of action is not a limitation in the claims. However, Applicants respectfully submit that they are not required to recite the mechanism of action in the claims as the prior art cited by the Examiner does not disclose or suggest that cancerous tumors could be treated anywhere but locally by the administration of SOD.

As discussed previously, it had been thought that SOD inhibited the anti-cancer activity of conventional treatments such as x-ray irradiation, anti-cancer drugs and anti-tumor activity of immune leukocytes because reactive oxygen generated by x-ray irradiation, various anti-cancer drugs and macrophages in NK cells kill cancer cells by releasing reactive oxygen. The present invention is especially surprising in light of the common knowledge of the prior art.

The instant invention is directed to a method of inhibiting the malignant progression of the tumor and a method for inhibiting the metastasis of a tumor in a subject. Tumorigenesis, which means the changing from a normal cell to a tumor cell, is the result of many steps of genetic mutations, also called "malignant transformation", "malignant progression", or simply "progression". Progression of a tumor takes place during the process of tumorigenesis as well as the process of treating the tumor. As such, the present invention, which inhibits the malignant progression of the tumor, is especially important in the treatment of cancer and the efficacy of the present invention in inhibiting the malignant progression of a tumor and inhibiting the metastasis of a tumor is clearly supported by the objective evidence of record in the present application and a further enclosed Declaration Under 37 CFR 1.132.

In the Example contained on pages 6-10 of the present specification, the QR-32 tumor cell line, which is a benign tumor, was used in order to test the efficacy of the present invention. This is shown in Table 1, Experiment B, left column at Lung-colonizing ability, where it is shown that the QR-32 tumor cell line did not produce lung metastasis in 10 mice after intravenous injection. This shows that the original tumor cell line, QR-32, was benign.

The QR-32 cells were subcutaneously implanted with gelatin sponge into mice in order to induce tumor formation. In fact, in the mice that were administered the physiological saline solution, 8 out of 9 mice developed tumor modules at the injection site. Conformance that the tumor takes were the result of malignant transformation of the benign QR-32 cells was confirmed by the next experiments which showed that the cells recovered from the tumor nodules at the injected sites where gelatin sponge had metastasizing ability. As shown in Table 1, Column 3 from the left, the incidence of lung metastasis (number of mice with lung metastasis/number of mice tested) was 0/10, 16/19 and 5/12 respectively for each of the benign tumor QR-32, total of saline treated QRs P-1 to QRs P-5 and total of SOD-G treated QRs P/SOD-G-1 to QRs P/SOD-G-3. A significant difference $p < 0.05$ was observed by statistical analysis of the incidences of the saline-treated group, 16/19, to that of the SOD-G treated group, 5/12. This proves that the SOD-G prevented the malignant progression of the tumor.

Enclosed herewith is a Declaration Under 37 CFR 1.132 which presents additional data in showing the inhibition of malignant progression of tumors by SOD-G. As illustrated in Column 5 of Table 2 in the Declaration, there was a significant reduction in the number of cell lines established/number of tumors tested (%) in the SOD-G treated group, 7/17 (41%), compared with that of the saline-treated group, 15/19 (79%) and the gliadin-treated group, 15/18 (83%).

The inhibition of the lung metastatic ability of SOD-G treated tumor cell lines (QRs P/OK) is shown in Table 3 of the

enclosed Declaration Under 37 CFR 1.132 and is compared with the saline-treated group (QRs P) and the gliadin-treated group (QRs P/GD). The number of lung metastatic nodules in the group treated with SOD-G was 1.6 ± 3.9, which was significantly lower than that of the saline-treated group, 50.1 ± 61.5, and the gliadin-treated group, 41.5 ± 55.9. As such, the inhibition of the metastasis of the tumor is clearly shown by this data.

Table 4 in the enclosed 37 CFR 1.132 Declaration compares the SOD-G of the present invention with purified melon SOD for tumorigenicity and metastasis inhibition. As shown in column 2 of Table 4, the mice that were given SOD-G had a much lower occurrence of tumors (44%) than the mice that were administered the purified melon SOD (100%).

All six cell lines established from tumor nodules removed from SOD-treated mice were metastatic, as shown in Column 5 of Table 4. In contrast thereto, none of the cell lines removed from the tumor nodules of the SOD-G treated mice were metastatic. This clearly establishes the unexpectedly superior properties of SOD-G of the present invention over SOD inhibiting tumorigenicity and metastasis of cancer cells.

As discussed above, it is respectfully submitted that the patentability of the presently claimed invention clearly has been established. The Examiner is respectfully requested to reconsider the present application and to pass it to issue.

Respectfully submitted,

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Encl: Declaration Under 37 CFR 1.132
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IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: Futoshi OKADA et al

For: ANTI-TUMOR AGENT

Serial No.: 10/655 567 Group: 1651

Confirmation No.: 6434

Filed: September 4, 2003 Examiner: Kosson

Atty. Docket No.: Furuya Case 1407

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

I, Hiroshi SHIONOYA, hereby declare as follows:

I am one of the co-inventors of the invention described and claimed in application Serial No. 10/655 567, filed on September 4, 2003.

I hereby incorporate by reference herein the contents of the Example contained on pages 6-10 of the above-identified application.

I have carried out additional tests to illustrate the unexpected properties of the anti-tumor agent of the present invention in inhibiting the malignant progression of a tumor.

The procedures of the Example contained on pages 6-10 of application Serial No. 10/655 567 to generate additional test data showing the efficacy of the present invention in inhibiting the malignant progression of a tumor. Additional mice were co-implanted with QR-32 cells and a gelatin sponge. The mice were then treated with a physiological saline solution, gliadin and SOD-G of the present invention. The results are shown in Table 2 below.

Table 2 Inhibition of inflammation-promoted acquisition of metastastic ability of QR-32 cells by SOD-G

[a] Experiment A: A: Tumorigenicity of QR-32 cells co-implanted with gelatin sponge in mice					[c] Experiment B: Characteristics of the arising tumor lines				
						Lung-colonizing ability		Other metastasis sites	
Treated with	No. of mice with tumor take/no. of mice treated(%) Exp. 1	Exp. 2	Total	[b] No. of cell lines established/ no of tumors tested(%)	Cell lines established From the arising tumor	Incidence (No. of mice with lung metastasis/no. of mice tested)	No. of lung with metastastic nodules	Incidence (No. of mice with other metastasis/ no of mice tested .	Sites (Incidence)
-	-	-	-	-	QR-32	0/10	0,0,0,0,0,0,0,0,0,0	0/10	None
Saline	8/9 (89%)	7/10 (70%)	15/19 (79%)	15/19 (79%)	QRsP/-1	3/3 **[d]	1,3,14	0/3 NS	None
					QRsP/-2	3/3 **	8,13,20	0/3 NS	None
					QRsP/-3	4/4 **	3,8,14,>150	1/4 NS	O (1/4)
					QRsP/-4	3/4 **	0,1,3,35	0/4 NS	None
					QRsP/-5	4/5 **	0,2,8,15	1/5 NS	O (1/5)
					QRsP/-6	4/4 **	8,43,>150,>150	2/4 NS	O (2/4)
					QRsP/-7	4/4 **	8,11>150,>150	2/4 NS	O (2/4)
					QRsP/-8	4/4 **	>150,>150,>150,>150	0/4 NS	None
					QRsP/-9	4/4 **	16,48,51,62	0/4 NS	None
					Total	33/35 (94%)		6/35 (17%)	
Gliadin	8/8 (100%)	7/10 (70%)	15/18 (83%)	15/18 (83%)	QRsP/GD-1	4/4 **	2,2,4,17	0/4 NS	None
					QRsP/GD-2	4/4 **	5,8,12,14	0/4 NS	None
					QRsP/GD-3	2/4 NS	0,0,3,8	0/4 NS	None
					QRsP/GD-4	3/4 *	0,5,7,22	1/4 NS	O (1/4)
					QRsP/GD-5	3/4 *	0,3,6,12	0/4 NS	None
					QRsP/GD-6	4/4 **	2,5,6,7	1/4 NS	O(1/4)
					QRsP/GD-7	3/3 **	16,>150,>150	1/3 NS	O (1/3),A(1/3)
					QRsP/GD-8	4/4 **	25,58,192,>150	3/4 *	O (2/4),LN(1/4)
					QRsP/GD-9	4/4 **	58,73,>150,>150	0/4 NS	None
					QRsP/GD-10	4/4 **	42,49,123,>150	0/4 NS	None
					Total	35/39 (90%)		5/39 (13%)	
SOD-G	4/7 (57%)	6/10 (60%)	10/17 (59%)	7/17 (41%) p<0.05 vs saline	QRsP/OK-1	0/4 NS	0,0,0,0	0/4 NS	None
					QRsP/OK-2	3/4 *	0,2,3,18	0/4 NS	None
					QRsP/OK-3	2/4 NS	0,0,5,7	0/4 NS	None
					QRsP/OK-4	0/4 NS	0,0,0,0	0/4 NS	None
					QRsP/OK-5	1/4 NS	0,0,0,8	0/4 NS	None
					QRsP/OK-6	0/4 NS	0,0,0,0	0/4 NS	None
					QRsP/OK-7	1/4 NS	0,0,0,8	0/4 NS	None
					Total	7/28 (28%) p<0.01 vs saline		0/28 (0%) p<0.05 vs saline	

Table Legends

a: In Experiment A, 1 x 10^6 QR-32 tumor cells were co-implanted with gelatin sponge in mice to which oxykine, gliadin and saline as a control had been orally administered once daily from 2 days before tumor implantation and following 4 weeks. Doses of Oxykine and SOD were 10mg/kg (=10 U/kg as SOD activity) and equivalent amount of gliadin (0.2mg/kg). respectively in 0.2ml saline

b: Culture cell lines are separately established from tumors which had arisen in each mouse.

c: In Experiment B, 1 x 10^6 cell of each cell lines were intravenously injected into mice. Twenty-five days later, the mice were sacrified and the metastatic nodules on the lung surface were counted macroscopically.

d: $p<0.01$ vs incidence of QR-32 (0/10)

e: $p<0.05$ vs incidence of QR-32 (0/10)

f: not significant vs incidence of QR-32 (0/10)

g: O: ovary, A: ascites, LN: Lymph node

Tumor cell lines arising from the mice having the co-implanted QR-32 cells and a gelatin sponge and treated with the physiological saline solution, gliadin and SOD-G of the present invention were tested for lung metastatic ability. The results are shown in Table 3.

Table 3 Inhibition of lung metastatic ability of QRsP/OK tumor lines

Cell lines	Metastatic incidence (%)	Lung weight (g)	No. of lung metastatic nodules	Median value	Range	Percent reduction
QRsP	33/35 (94)	0.45±0.46	50.1±61.5	12	0-150	0
QRsP/GD	35/39 (90) a	0.37±0.39 b	41.5±55.9 a	15	0-150	17
QRsP/OK	7/28 (25) a	0.18±0.02 b	1.6±3.9 a	0	0-18	97

QRsP/OK tumor lines, shown in Table 2, are cell lines established from the arising tumor after subcutaneous transplantation of benign tumor cell of QP-32 with gelatin sponge into mice treated with SOD-G.
Cell lines QRsP and QRsP/GD are those which established from mice treated saline and gliadin, respectively.

a:$p<0.001$, b:$p<0.005$ as compared to QRsP/GD, Glyadin treated group of mice.

In Table 4 shown below, additional mice were co-implanted with QR-32 cells and a gelatin sponge. The mice were then treated with a physiological saline solution, SOD and SOD-G of the present invention. The SOD was purified melon SOD per se. The dose of SOD was 10 U/kg which was equivalent to 10 mg/kg of SOD-G. The results are shown below in Table 4.

Table 4 Inhibition of inflammation-promoted acquisition of metastatic ability of QR-32 cells by SOD-G

A: Tumorigenicity of QR-32 cells co-implanted with gelatin sponge in mice			Characteristics of the arising tumor lines				
				Lung-colonizing ability		Other metastasis sites	
Treated with	No. of mice with tumor take/no. of mice treated(%) Exp. 3	No. of cell lines established/no of tumors tested(%)	Cell lines established From the arising tumor	Incidence (No. of mice with lung metastasis/no. of mice tested)	No. of lung with metastatic nodules	Incidence (No. of mice with other metastasis/no of mice tested .	Sites (Incidence)
			QR-32	0/10	0,0,0,0,0,0,0,0,0,0	0/10	None
Saline	7/8 (88%)	6/7 (86%)	QRsP/-10	3/3 ** a	3,8,150	0/3	None
			QRsP/-11	3/3 **	8,13,20	0/3	None
			QRsP/-12	3/3 **	10,14,>150	1/3	[O* (1/3)]
			QRsP/-13	2/3 * b	0,3,35	0/3	None
			QRsP/-14	3/3 **	2,8,15	1/3	[O (1/3)]
			QRsP/-15	1/3 NS c	0,0,3	1/3	[O (1/3)]
			Total	15/18		3/18	
SOD	7/7 (100%)	6/7 (86%)	QRsP/SOD-1	3/3 **	31,38,>150	1/3	[O,(1/3)]
			QRsP/SOD-2	2/3 *	0,5,38	0/3	None
			QRsP/SOD-3	3/3 **	10,41,58	0/3	None
			QRsP/SOD-4	2/3 *	0,9,13	0/3	None
			QRsP/SOD-5	3/3 **	6,6,30	0/3	None
			QRsP/SOD-6	3/3 **	36,52,>150	1/3	[O(1/4)]
			Total	16/18		2/18	
SOD-G	4/9 (44%) p<0.05 vs SOD	4/9 (44%)	QRsP/OK-8	0/3 NS	0,0,0	0/3	None
			QRsP/OK-9	1/3 NS	0,0,3	0/3	None
			QRsP/OK-10	1/3 NS	0,0,7	0/3	None
			QRsP/OK-11	0/3 NS	0,0,0	0/3	None
			Total	2/12 p<0.01 vs SOD p<0.01 VS saline		0/12	

*: Ovary
a; p<0.01 vs incidence of QR-32 (0/10)
b; p<0.05 vs incidence of QR-32 (0/10)
c; not significant vs incidence of QR-32 (0/10)

DISCUSSION OF RESULTS

As can be seen by the data contained in Table 2, the mice that were administered the SOD-G of the present invention had a significantly lower occurrence, 41%, of tumors than the mice that were administered the physiological saline solution, 79%, or the gliadin, 83%.

Additionally, the tumor cells arising in the mice that were administered the SOD-G of the present invention had a greatly reduced metastatic ability, 97% reduction, than the tumor cells arising from the mice that were administered gliadin, 17% reduction, or the physiological saline solution, 0% reduction.

As illustrated in column 2 of Table 4, the mice that were administered the SOD-G according to the present invention had a much lower occurrence of tumors (44%) than the mice that were administered the physiological saline solution (88%) or the purified melon SOD (100%).

With respect to the metastatic ability of the QR-32 cells, as shown in column 5 of Table 4, all 6 cell lines established from tumor nodules removed from SOD-treated mice, QRsP/SOD-1 to QRsP/SOD-6, were metastatic. In contrast thereto, none of the 4 cell lines recovered from the tumor nodules of the SOD-G treated mice were metastatic ($p<0.01$). This establishes that SOD-G inhibited malignant progression and metastatic ability while SOD did not.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: Apr. 13, 2005

Hiroshi Shionoya

Hiroshi SHIONOYA